

- E1*
- (b) subculturing reddish epidermal callus to embryo induction medium comprising casein hydrolysate and  $\text{NH}_4^+$  and/or  $\text{NO}_3^-$  to form embryogenic callus;
  - (c) culturing said embryogenic callus on developmental medium containing an osmotic pressure increasing agent;
  - (d) culturing said embryogenic callus on maturation medium; and
  - (e) recovering poinsettia plants from said embryos.
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*Sub F2*

6. (Twice Amended) A method for producing transgenic poinsettia plants, comprising [the steps of]:

- E2*
- (a) incubating poinsettia plant tissue explants that produce reddish epidermal callus on auxin- and cytokinin-containing callus induction medium;
  - (b) culturing reddish epidermal callus on embryo induction medium comprising casein hydrolysate and  $\text{NH}_4^+$  and/or  $\text{NO}_3^-$  to form embryogenic callus;
  - (c)
    - (i) introducing an expression vector into said incubating embryogenic callus to produce transformed embryogenic callus, wherein said expression vector comprises a selectable marker gene and a second foreign gene, or
    - (ii) introducing two expression vectors into said incubating embryogenic callus to produce transformed embryogenic callus, wherein one of said expression vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene;

wherein the vector or vectors are introduced into the incubating embryogenic callus by co-incubating the callus with *Agrobacterium*

tumefaciens containing the vector or vectors, by microprojectile-mediated delivery of the vector into the callus, or by electroporation:

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- (d) culturing said transformed embryogenic callus on selection medium;
  - (e) culturing said transformed embryogenic callus containing embryos on developmental medium containing an osmotic pressure increasing agent;
  - (f) culturing said transgenic embryos on maturation medium; and
  - (g) recovering transgenic plants from said transgenic embryos.

17. (Amended) The method of claim 6, wherein the expression of said second foreign gene confers resistance to disease caused by an organism selected from the group consisting of virus, bacterium, and fungus [and insect].

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Sub 37 18. (Amended) The method of claim 17, wherein said second foreign gene disrupts the function of said virus, and wherein said virus-disrupting gene is selected from the group consisting genes encoding [of] viral coat protein, 2'-5' oligonucleotide synthetase, viral genome antisense RNA, and pokeweed antiviral protein.

19. (Amended) The method of claim 6, wherein said second foreign gene confers resistance to an insect, and wherein said insect resistance gene encodes a protein [is] selected from the group consisting of tryptophan decarboxylase, lectin, and *Bacillus thuringiensis* toxin

Sub 47 39. (Twice amended) A method for producing transgenic poinsettia plants, comprising [the steps of]:

- 4
- (a) incubating poinsettia plant tissue explants that produce reddish epidermal callus in auxin- and cytokinin-containing callus induction medium;

(b) culturing embryogenic callus produced on said callus induction medium in liquid NH<sub>4</sub><sup>+</sup> and/or NO<sub>3</sub><sup>-</sup> containing embryo induction medium;

(c) filtering the culture and culturing the filtrate in fresh liquid embryo induction medium;

(d) filtering the culture and culturing the filtrate on solid embryo induction medium;

(e) culturing embryos produced on said embryo development medium on maturation medium;

(f) culturing said embryos on callus induction medium;

(g) culturing epidermal callus produced on said callus induction medium on embryo induction medium to form embryogenic callus;

(h)

(i) introducing an expression vector into said embryogenic callus to produce transformed embryogenic callus, wherein said expression vector comprises a selectable marker gene and a second foreign gene, or

(ii) introducing two expression vectors into said embryogenic callus to produce transformed embryogenic callus, wherein one of said expression vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene;

wherein the vector or vectors are introduced into the incubating embryogenic callus by co-incubating the callus with *Agrobacterium tumefaciens* containing the vector or vectors, by microprojectile-mediated delivery of the vector into the callus, or by electroporation;

(i) culturing said transformed embryogenic callus on selection medium;

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- 24
- (j) culturing said transformed embryogenic callus containing embryos on developmental medium containing an osmotic pressure increasing agent;
  - (k) culturing said transformed embryos on maturation medium;
  - (l) recovering transgenic plants from said transgenic embryos.
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51. (Amended) The method of claim 39, wherein the expression of said second foreign gene confers resistance to disease caused by an organism selected from the group consisting of virus, bacterium, and fungus[, and insect].

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F5  
257 52. (Amended) The method of claim 51, wherein said second foreign gene disrupts the function of said virus, and wherein said virus-disrupting gene is selected from the group consisting genes encoding [of] viral coat protein, 2'-5' oligonucleotide synthetase, viral genome antisense RNA, and pokeweed antiviral protein.

53. (Amended) The method of claim 51, wherein said second foreign gene confers resistance to an insect, and wherein said insect resistance gene encodes a protein [is] selected from the group consisting of tryptophan decarboxylase, lectin, and *Bacillus thuringiensis* toxin

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F67 76. (Amended) The method of claim 73, wherein the expression of said second foreign gene confers resistance to disease caused by an organism selected from the group consisting of virus, bacterium, and fungus[, and insect].

26 77. (Amended) The method of claim 76, wherein said second foreign gene disrupts the function of said virus, and wherein said virus-disrupting gene is selected from the group consisting genes encoding [of] viral coat protein, 2'-5' oligonucleotide synthetase, viral genome antisense RNA, and pokeweed antiviral protein.

36 78. (Amended) The method of claim 70, wherein said second foreign gene confers resistance to an insect, and wherein said insect resistance gene encodes a protein [is] selected from the group consisting of tryptophan decarboxylase, lectin, and *Bacillus thuringiensis* toxin

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P7 101. (Amended) A method for *in vitro* regeneration of poinsettia plants comprising [the steps of]:

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MF
- [epidermal]
- (a) incubating poinsettia plant tissue explants that produce epidermal callus on auxin- and cytokinin-containing callus induction medium;
- [said embryogenic]
- (b) subculturing reddish epidermal callus to NH<sub>4</sub><sup>+</sup> and/or NO<sub>3</sub><sup>-</sup> containing embryo induction medium to form embryogenic callus;
- (c) culturing said embryogenic callus on developmental medium containing an osmotic pressure increasing agent;
- (d) culturing said embryogenic callus on maturation medium; and
- (e) recovering poinsettia plants from said embryos.

Please add the following claims:

110. The method of claim 6, wherein the expression of said second foreign gene confers resistance to an insect.

38 SUB  
P97 111. The method of claim 49, wherein the expression of said second foreign gene confers resistance to an insect.

112. The transgenic poinsettia plant of claim 73, wherein the expression of said second foreign gene confers resistance to an insect.